Report on Propionic Acid, Sodium Propionate, Calcium Propionate, Thiodipropionic Acid & Dilauryl Thiodipropionic Acid 1/28/74

Q 30

VOL. I

Q 30

FOOD AND DRUG LABORATORIES, INC.

REPORT

PROPIONIC ACID

CAS. REG. NO. 000079-09-4

SODIUM PROPIONATE

CAS. REG. NO. 000137-40-6

CALCIUM PROPIONATE

CAS. REG. NO. 004075-81-4

THIODIPROPIONIC ACID

CAS. REG. NO. 000111-17-1

DILAURYL THIODIPROPIONIC ACID

CAS. REG. NO. 000123-28-4

Submitted to:

GRAS Review Branch (BF-335)
Bureau of Foods
Food and Drug Administration
200 C Street, S. W.
Washington D.C., 20204

Attention:
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Date: January 28, 1974

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SUMMARY

In this review propionic acid, sodium propionate, calcium propionate, thiodipropionic acid, and dilauryl thiodipropionate are considered. Propionic acid is a member of the alaphatic monocarboxylic acid series; it is a liquid, has a strong odor and is somewhat corrosive. The propionates used in the food industry as antimicrobials are the sodium and calcium salts, which yield free acid in the pH range of the foods in which they are used.

Under federal regulation, propionates are generally recognized as safe for use in foods and no upper limits are imposed on usage, excepting bread, rolls, and cheese which come under standard of identity. Sodium and calcium propionate are limited to 0.32% of flour in white bread and rolls and to 0.38% in the corresponding whole wheat products; in cheese products they are limited to 0.3% of the cheese. The propionates are not mentioned in the food additive regulations of France, West Germany, Greece, Portugal, and Turkey but they are included in those of other western European countries for which information concerning them is at hand. The major usages of propionates are to prevent mold and rope in baked goods and mold in certain types of cheese (407a).

Propionic acid is used a an esterfying agent in the production of cellulose, other propionates are used as mold inhibitors, in the manufacture of ester solvents, fruit flavors, and perfume bases (234a).

Sodium propionate has been used as a topical antifungal agent and in chronic bacterial conjunctivitis and bletharitis. In the veterinary field, it is used in the prevention of bovine ketosis, dermatosis, wound infections, conjunctivitis, vaginitis, and otitis (234a).

Calcium propionate has been used as an inhibitor of molds and other micro-organisms in foods, tobacco, and in the production of butyl rubber to improve processibility and scorch resistance, while medically it has been used as an antifungal agent in dermatology (234a).

Thiodipropionic acid is used as an antioxidant for soap products, as an esterfier in plasticizers and lubricants and is proposed for use in edible fats, oils and other foods (234a).

The acute toxicities for various species and for various routes of administration are shown in Table 1 of the text for propionic acid, sodium propionate, calcium propionate, thiodipropionic acid and dilauryl thiodipropionate. In acute rabbit eye irritation studies, the edema produced by propionate was quite similar to the edema produced with the same molar concentration of hydrochloric acid (196).

The addition of propionate to diets fed to varying species at levels of 24% or below did not affect growth rate in the adult animals but did affect the growth rate and physical well-being of weanling rats fed a diet containing 24% (231, 461, 571, 85).

When sodium propionate was fed to rats as 75% of the diet for 32 weeks, a depression of growth during the first week of feeding was observed, but it did not influence mortality and did not produce any detectable effects on hemoglobin levels, organ weights or histopathology of the tissues (412).

A diet containing 10% sodium propionate fed twice daily for 50 days increased the amount of fat in sheep at slaughter, indicating that they converted digestible energy to weight gains considerably better than did the controls (85).

In vitro studies utilizing human skin and fetal lung cells and strain L cells showed that a concentration of 10 mg% sodium propionate was toxic to these cell lines, but 1 mg% had no significant effect. Propionic acid and thiodipropionic acid showed a great anticarcinogenic activity against the growth of transplanted tumor cells in mice (347, 571).

In intestinal sections, propionic acid has an inhibitory effect on the transfer of fatty acids; the concentrations necessary to produce the inhibition decrease with the increase in length of the fatty acid chain. In the ingesta of chickens, fatty acids are completely removed from the ingesta before it reaches Meckel's diverticulum; in mice the preferential movement of propionic acid is in the jejunum rather than in the ileum (55, 35).

In Vitamin B_{12}-deficient humans, the injected radioactivity of propionates found in urinary methylmalonic acid is small; 20 to 50% of the propionate administered is the daily turnover in normal patients and 20% in Vitamin B_{12}-deficient patients. The fate of the remainder is unknown. Studies show that propionic acid is the second most effective of the fatty acids in penetrating the red blood cells, thus producing hemolysis, and that increasing the osmotic concentration of the outside fluid retards hemolysis.

The electroencephalogram patterns of the adult rabbits which were injected i.v. with a propionate solution showed changes in the EEG pattern from a resting type to one of sleep. These patterns were very similar to those observed under deep sodium penabarbatol anesthesia (372).

CHEMICAL INFORMATION

I. Nomenclature

A. Common names (234a)

Propionic acid-

Sodium propionate-

propionic acid sodium salt

Calcium propionate-

propionic acid calcium salt

Thiodipropionic acid-

No other common names were encountered in the literature searched.

Dilauryl thiodipropionate acid

No other common names were encountered in the literature searched.

B. Chemical names (234a, 176a)

Propionic acid-

propionic acid, methylacetic acid, ethylformic acid

Sodium propionate-

No other chemical name encountered in the literature searched.

Calcium propionate-

No other chemical name encountered in the literature searched.

Thiodipropionic acid-

3, 3'-thiodipropionic acid, β, β-thiodipropionic acid, thiodihydracrylic acid, diethylsulfide 2, 2'-dicarboxylic acid.

Dilauryl thiodipropionate acid

No other chemical name encountered in the literature searched.

C. Trade Names (234a, 258a)

Propionic acid-

No other trade names were encountered in the literature searched.

Sodium propionate-

Mycoban

Calcium propionate-

Mycoban

Thiodipropionic acid-

β, β'thiodipropionic acid

Dilauryl thiodipropionate acid

b, b'dilauryl thiodipropionate, Milban F.

D. Chemical Abstracts Services Unique Registry Number

Propionic acid

000 070-09-4

Sodium propionate

000137-40-6

Calcium propionate

004075-81-4

Thiodipropionic acid

000 111-17-1

Dilauryl thiodipropionate

000 123-28-4

II. Empirical Formula (234a, 105a)

Propionic acid

$C_3H_6O_2$

Sodium propionate

$C_3H_5NaO_2$

Calcium propionate

$C_6H_{10}CaO_4$

Thiodipropionic acid

$C_6H_{10}O_4S$

Dilauryl thiodipropionate

$C_{30}H_{58}O_4S$

III. Structural Formula (234a, 407a, 105a)

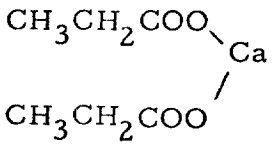
Propionic acid

CH_3CH_2COOH

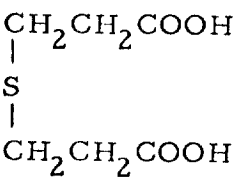
Sodium propionate

CH_3CH_2COONa

Calcium propionate



Thiodipropionic acid



Dilauryl thiodipropionate

$(C_{12}H_{25}OOCCH_2CH_2)_2S$

IV. Molecular Weight (234a, 105a)

Propionic acid

74.08

Sodium propionate

96.07

Calcium propionate

186.22

Thiodipropionic acid

178.20

Dilauryl thiodipropionate

514.85

V. Specifications

A. Chemical (234a)

Propionic acid

C	48.64%
H	8.16%
O	43.20%

Sodium propionate

C	37.50%
H	5.25%
O	33.31%
Na	23.94%

Propionic acid 77.11%

Calcium propionate

C	38.70%
H	5.41%
O	34.37%
Ca	21.52%

Thiodipropionic acid

C	40.44%
H	5.66%
O	35.91%
S	17.99%

Dilauryl thiodipropionate

C	70.0%
H	11.3%
O	12.4%
S	6.2%

B. Food Grade (105a)

Propionic acid

Assay. Not less than 99.5 percent of $C_3H_6O_2$.
Distillation range. Between 138.5° and 142.5°.
Specific gravity. Between 0.993 and 0.997 at 20°/20°.
Limits of Impurities
Aldehydes (as propionaldehyde). Passes test (limit about 0.05 percent).
Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Heavy metals (as Pb). Not more than 10 parts per million (0.001 percent).
Nonvolatile residue. Not more than 0.01 percent.
Readily oxidizable substances (as formic acid). Passes test (limit about 0.05 percent).
Water. Not more than 0.15 percent.

Sodium propionate

Assay. Not less than 99.0 percent of $C_3H_5NaO_2$ after drying.

Limits of Impurities

Alkalinity (as Na_2CO_3). Passes test (about 0.15 percent).
Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Heavy metals (as Pb). Not more than 10 parts per million (0.001 percent).
Iron. Not more than 30 parts per million (0.003 percent).
Water. Not more than 1 percent.

Calcium propionate

Assay. Not less than 98.0 percent of $C_6H_{10}CaO_4$, calculated on the anhydrous basis.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Fluoride. Not more than 30 parts per million (0.003 percent).
Heavy metals (as Pb). Not more than 10 parts per million (0.001 percent).
Insoluble substances. Not more than 0.2 percent.
Magnesium (as MgO). Passes test (about 0.4 percent).
Water. Not more than 5 percent.

Thiodipropionic acid

No Food Grade information was encountered in the available literature.

Dilauryl thiodipropionate acid

Assay. Not less than 99.0 percent of $C_{30}H_{58}O_4S$.
Solidification point. Not below 40°.

Limits of Impurities

Acidity (as thiodipropionic acid). Not more than 0.2 percent of $C_6H_{10}O_4S$.
Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent).
Lead. Not more than 10 parts per million (0.001 percent).

C. Official Compendia

Food Chemical Codex

VI. Description

A. General Characteristics (105a, 234a)

Propionic acid

An oily liquid having a slightly pungent, rancid odor. It is miscible with water and with alcohol and various other organic solvents.

Sodium propionate

Colorless, transparent crystals or a granular crystalline powder. It is odorless, or has a faint acetic-butyric odor. It is deliquescent in moist air.

Calcium propionate

White crystals or crystalline solid, possessing not more than a faint odor of propionic acid.

Thiodipropionic acid

Nacreous leaflets from hot water.

Dilauryl thiodipropionate acid

White crystalline flakes having a characteristic sweetish, ester-like odor.

B. Physical Properties (176a, 105a, 234a)

Propionic acid

melting point, °C	−22
boiling point, °C	141.1
vapor pressure, mm Hg, at 28.0°C	5
at 52.0°C	20
at 85.8°C	100
at 122.0°C	400
specific gravity, 20/4°C	0.992
20/20°C	0.9952
refractive index, n_D^{20}	1.3874
viscosity at 25°C, cP	1.035
surface tension in air at 20°C, dyn/cm	26.70
coefficient of cubical expansion at 0–133°C/°C	1.102×10^{-3}
critical temperature, °C	337.6
critical pressure, atm	53.0
specific heat at 20–137°C, cal/g	0.726
heat of fusion at melting point, cal/g	23.4
heat of vaporization at 139.3°C, cal/g	98.9
heat of combustion of liquid, cal/g	4931.5
flash point, Cleveland open cup, °F	150
weight at 20°C, lb/gal	8.28

Sodium propionate

One gram is soluble in about 1 ml of water at 25°, in about 0. 65 ml of boiling water, and in about 24 ml of alcohol. The pH of a 1 in 10 solution is between 8. 0 and 10. 5.

Calcium propionate

One gram dissolves in about 3 ml of water. The pH of a 1 in 10 solution is between 8 and 10. Slightly soluble in methanol, ethanol; practically insoluble in acetone and benzene.

Thiodipropionic acid

m. p. 134°C

Kat 25°C 7.8×10^{-5}

One gram dissolves in 26. 9 ml H_2O at 26°C. Freely soluble in hot water, alcohol, acetone.

Dilauryl thiopropionate

Insoluble in water.
Soluble in most organic solvents.

C. Stability in Containers * (176a, 407a, 105a)

Propionic acid

Handle as any organic chemical of comparable flash point and vapor pressure. Uncoated steel containers are not recommended.

Sodium propionate

Emits acrid fumes at high temperatures.
Presents little hazard in storage.

Calcium propionate

Emits acrid fumes at high temperature, protect from humidity, indefinite shelf-life.

Thiodipropionic acid

No stability information encountered in the literature searched.

Dilauryl thiopropionate

Store in well-closed containers.

*Private communication from unidentified source provided by the FDA.

VII. Analytical

Compound:	Propionic acid
Title:	Gas chromatographic determination of propionic, sorbic, and benzoic acids in rye bread and margarine (123a).
Principle determination step:	Flame ionization dector
Principle separation technique:	Extraction and mixed carbowax, terephthalic acid on chromosorb W(AW-DMCS).
Principle analytical reagent:	Valeric acid
Moiety measured:	Unknown

Compound:	Sodium propionate
Title:	Separation and identification of sodium salts of acetic, propionic, butyric and valeric acids by paper chromatography (475).
Principle determination step:	Color
Principle separation technique:	Silicic acid column
Principle analytical reagent:	Methyl red, bromothyml blue in formalin at pH 5. 2.
Moiety measured:	Acid group.

Compound:	Propionic acid
Title:	Determination of volatile free fatty acids of human blood (221).
Principle determination step:	Flame ionization
Principle separation technique:	Extraction, gas liquid chromatography.
Principle analytical reagent:	Chloroform-methanol-sulfuric acid mixture.
Moiety measured:	Unknown

Compound:	Propionic acid
Title:	Identification of acetic, propionic and sorbic acid in bakery products by thin layer chromatography (346).
Principle determination step:	Color
Principle separation technique:	Distillation, thin layer chromatography.
Principle analytical reagent:	H_3PO_4, bromcresol purple.
Moiety measured:	Acid group

Compound:	Thiodipropionic acid
Title:	The multi-determination of antioxidants in lard (567).
Principle determination step:	Infrared spectrophotometry
Principle separation technique:	Vacuum sublimation, gas chromatography.
Principle analytical reagent:	Diazomethane
Moiety measured:	All IR absorbing bonds.

Compound:	Dilauryl thiodipropionate
Title:	Determination of DLTDP and other antioxidants by a modified sublimation technique (583).
Principle determination step:	Spectrophotometry
Principle separation technique:	Sublimation, gas liquid chromatography
Principle analytical reagent:	1% Apiezon N on gas Chrom Q.
Moiety measured:	All IR absorbing bonds.

Calcium propionate: No method specifically for calcium propionate was found in the literature searched. However, the procedure of Young et al (475) implies use for propionates in general.

VIII. Occurrence and Levels Found in:

A. Plants (248a, 234a)

Propionic acid

Apples, strawberries, tea, violet leaves.
Wood distillate. Bacterial action on many materials.

Sodium propionate

No occurrences or levels found in plants encountered in the literature searched.

Calcium propionate

No occurrences or levels found in plants encountered in the literature searched.

Thiodipropionic acid

No occurrences or levels found in plants encountered in the literature searched.

Dilauryl thiodipropionate

No occurrences or levels found in plants encountered in the literature searched.

B. Animals (176a, 234a, 407a)

Propionic acid

Stomach of ruminants
Dairy products - small amounts

Sodium propionate

No occurrences or levels found in animals encountered in the literature searched.

Calcium propionate

No occurrences or levels found in animals encountered in the literature searched.

I Acute Toxicity

Table 1

Substance	Animal and Strain	Number	Route	LD_{50} (mg/kg body wt.)	Ref.
Propionic acid	Mice	Minimum 6 groups x 10	i. v.	625 ± 33	(260)
	Mice		n. a.[1]	1, 370 ± 150	(314)
	Rats		n. a.[1]	1, 510 ± 250	(314)
	Rats		oral	4, 290 (10% sol.)	*
	Rats		oral	2, 600	(422a)
	Rabbits		n. a.[1]	1, 900	(314)
	Rabbits		skin	0. 5 ml/kg (500 mg/kg)	*
Sodium propionate	Mice		n. a.[1]	7, 800	(314)
	Mice (DD-strain)		i. v.	1, 380	(413)
			i. p.	2, 250	(413)
			i. m.	3, 200	(413)
			oral	5, 100	(413)
	Rats		n. a.[1]	8, 150	(314)
	Rabbit		n. a.[1]	6, 750	(314)

[1] n. a. = not available

*Private communication from unidentified source provided by FDA.

Table 1 (cont'd)

Substance	Animal and Strain	Number	Route	LD_{50} (mg/kg body wt.)	Ref.
Calcium propionate	Mice (DD-strain)		i. v.	580	(413)
			i. p.	720	(413)
			i. m.	1, 020	(413)
			oral	3, 340	(413)
	Rats		oral	5, 160 (4, 170-6, 380)	(546)
Thiodi-propionic acid	Rats		n. a.[1]	>2, 000	(566a)
Dilauryl thiodi-propionate	Rats		n. a.[1]	>2, 500	(422a)

Morphinized male albino rabbits weighing approximately 2 kg were used in eye irritation studies to observe the effect of chemical configuration on the edema-producing potency of acids and other compounds. The edema produced by propionate was quite similar to the amount produced with hydrochloric acid of the same molar concentration (196).

Acclimatization experiments were conducted in which the organisms were grown in nutrient broth containing 0.8% sodium propionate or sulfacetamide sodium. It was then sub-cultured for 4 days in broth with 1.25% of the separate drugs and the resultant cultures were used to inoculate broth containing various concentrations of the compounds. The results show that the organisms did not become appreciably resistant to sodium propionate. A test for synergism or antagonism was conducted using 2% sodium propionate + 2% sulfacetamide sodium. The results obtained with staphylococcus aureus suggest that antagonism does exist between the sodium propionate and sulfacetamide sodium preparations on local infections when propionate is applied topically to the eyes, ears, nose, vagina, vagina skin and other tissues (415).

Four-week-old white rats were used in a 4- to 5-week study of the toxicity of several food-preserving agents such as sodium propionate and calcium propionate. Weight gain was used as the indication of toxicity produced in animals fed diets containing 1 or 3% calcium or sodium propionate; neither additive affected the amount of weight gain (503).

To compare the utilization of energy, diets containing 24% acetate, propionate, or butyrate with glucose and starch were fed to weanling and adult female rats for periods of 5 or 20 days, respectively. Feeding of the propionate rations to the weanling animals was discontinued after 5 days due to high mortality and negative weight gain; the adult animals survived 20 days, but rough hair coats, reddened paws and extreme excitability were observed in all. Substitution of the propionate in the diet did not affect digestibility, metabolizability or energy measurements, but did interfere with normal metabolic function to the extent that it could not be fed to the younger rats and presented the aforementioned problems in the adults (231).

A series of studies were conducted to determine the fungicidal, fungistatic and antifermentative properties of sodium propionate. In the first study of the series, albino rats weighing approximately 200 g were given 100 mg/kg of propionate by hypodermic injection for a period of 5 weeks, while another group received 100 mg/kg by the same route of administration. Thereafter, there was a weekly dosage increase of 50 mg/kg until 350 mg/kg was reached. The results obtained show that there were no mortalities nor were there any noticeable variations in the weight of the treated animals at any of the dose levels when compared with the controls. After 4 weeks, there was a slight local loss of hair in the animals receiving the incremental doses, and in the 5th and 6th weeks, small subcutaneous abscessula protuberances

formed which may have been due to the large quantity of propionate administered. Analysis of the urine revealed that the volatile products of acid character underwent positive and negative variations of 3% in the control groups, whereas in the 2 medicated groups described, the same content increased by 11% and 16.5%, respectively. This finding led the authors to believe that part of the sodium propionate is eliminated through the urine in the form of volatile components without undergoing any preventive block within the organism. The second experiment of this series on the fungicidal activity of sodium propionate in vitro revealed that a .0125% to 2% solution of sodium propionate is one of the potent inhibitors of bacteria and fungi responsible for local and more serious infections. In the third series of experiments, apple juice, sweet filtrate of grape and orange juice were bottled and stored at temperatures between 20° and 30°. Either 0.2, 0.4, 0.6, or 0.8% by weight of sodium propionate was added to each group of 2 juices, and the samples were stored for approximately 10 months. The results show that 0.2, 0.4, and 0.6% concentrations would block fermentation for 5, 11, and 21 days, respectively, but would exert almost no action after that time (461).

Weanling pigs were administered propionic acid or calcium propionate, (to give 0.8% propionate), in order to determine the effect on performance and bacterial flora. Treatments were applied for 4 weeks, and performance was also measured for a further period of 8 weeks after treatment had ceased. In a second study, a pig from each of 9 litters was

slaughtered for examination before treatments were applied in order to obtain bacterial baseline data. The remaining animals were placed on the treatment as described above for a period of 4 weeks, after which samples for bacterial examination were taken from the duodenum and jejunum. These studies showed that after 4 weeks, there was no significant difference in the growth rate of the pigs, and that bacterialogically, both substances tested were effective in controlling hemolytic Escherichia coli and reducing non-hemolytic Escherichia coli in the duodenum and jejunum (57).

A preliminary feeding trial of 3-weeks duration indicated that there was little difference in acceptability of several diets by weanling rats. Therefore, a long-term study was conducted in male and female rats to determine the chronic toxicity of bread additives. The findings showed that the baked bread product, (5% concentrations of propionate were added to bread ingredients before baking), fed ad libitum to rats as 3/4 of the diet for a one-year period, did not manifest any toxic effects. A follow-up study was conducted in which animals were fed a diet containing 75% sodium propionate for 32 weeks. It was found that sodium propionate at this high level caused a depression of growth during the first weeks of feeding, but did not influence mortality and produced no detectable effect on hemoglobin levels, organ weights, or histopathology of the tissues (412).

Mixed-strain male and female albino rats, weighing 50 to 80 g, were fed a diet containing 5 to 10% propionic acid to determine the

production of gastric lesions by these diets. One rat on the propionic acid diet died early in the experiment, and the remaining 4 were sacrificed after 110 days. In 3 or 4 cases it was found that umbilicate or warty lesions were evidenced in the forestomach, while the remaining one showed no gastric changes (237).

Male and female shorn lambs weighing 23 to 35 kg were fed a ration containing 10% sodium propionate twice daily for 50 days ad libitum. It was found that neither rate of body weight gain or feed required/unit gain was affected by incorporating 10% sodium propionate in the feed. At slaughter, it was found that the animals fed the propionate-containing diets were considerably higher in fat than the controls indicating that they converted digestible energy to weight gain considerably more than did the controls (85).

A study utilizing human skin and fetal lung cells and strain L cells was conducted to determine the effects of sodium salts and certain monocarboxylic acids on established cell lines. The results revealed that at a concentration of 10 mg%, sodium propionate was toxic to all cell lines tested, but had no significant effect at a 1 mg concentration (442).

Walter Reed-Carworth Farm rats in their first gestation were administered a total dose of 0.5 g thiodipropionic acid during a 22-day diet period. On the 22nd day, the young were Caesarean delivered, and both horns of the uterus were excised and carefully

microscopically for resorption sights in order to determine if certain anti-oxidants influenced resorption in rats. The results show that, of 10 litters, there were 128 implantations, 119 were fetuses, and 7% were resorptions (575).

In vitro anti-tumor studies were carried out with 6 C3HED lymphosarcoma cells. Ehrlichascites carcinoma and TA3 mammary carcinoma were injected intraperitoneally in C3H mice or Connaught mice weighing 20 to 22 g. The washed tumor cell suspension was added to 1 ml of Fink's solution containing 1.6 mg of propionic acid, and 0.5 ml of this suspension was administered intraperitoneally to mice. The results show that the survival time of the test animals was significantly longer than the control animals; after 90 days, 2 of the 10 animals medicated were alive (347).

The inhibition of tumor growth with antimetabolites of the hexose monophosphate pathway intermediates was studied in transplantable Yoshida ascite sarcoma cells in rats and fibrosarcoma cells in Swiss mice. Thiodipropionic acid was injected intraperitoneally 24 hours after the transplantation of the Yoshida sarcoma cells in rats, while the compound was injected subcutaneously near the solid fibrosarcoma in the mouse on the 6th day after transplantation. Thiodipropionic acid completely destroyed the sarcoma cells after 10 days of treatment, and the animals lived a normal life span without any trace of malignancy, while the corresponding control animals died within 10 days (571).

Male Wistar rats were kept at 25°C for 10 days and on the 11th day were given 1 g of tripropionin and 1.9 g of dextrose containing -1-C_{14}. The rats were then placed in a metabolism cage, and their respiratory CO_2 was measured for 24 hours, after which the animals were sacrificed and head and carcass were analyzed for lipids. In a second study, animals were injected intraperitoneally with 1 ml of saline containing -1-C_{14} sodium propionate. They were placed at once in wire cages, either fed or fasted, were sacrificed after 24 hours, and the lipids were isolated and analyzed for C_{14}. The results showed that in the animals medicated with labeled sodium propionate by stomach tube, 75% of the C_{14} content appeared in the respiratory CO_2 during the 24-hour experiment. In the animals which were administered labeled propionate intraperitoneally, very little of the C_{14} appeared in the body fat. A third study showed that the rat liver was able to convert large amounts of acetate into fatty acids but was unable to convert any of the carboxyl or propionate into long-chain fatty acids. In the study of the adipose tissue, it was evident from the position in which the propionates were labeled that propionate 2 or 3 carbons are far better precursors of long-chain fatty acids than is the carboxylcarbon (226).

A section of rat jejunum approximately 30 cm long was inverted to form a sack which was suspended in 15 to 50 ml of saline solution in order to study the transfer of short-chain fatty acids by the intestines. Transfer experiments, to measure changes in the mucosa,

and metabolism experiments to estimate the total amount of fatty acids disappearing during the course of the experiment were conducted using propionic acid. Concentrations of propionic acid added to the baths ranged from 5 to 80 mm. It was found that propionic acid was transferred against the concentration gradient, that high concentrations of propionic acid had an inhibitory effect on transfer of the fatty acid, and that the concentration necessary to cause this inhibition decreased with increasing length of the fatty acid chain (22).

Another study, by different authors using the same techniques as above but utilizing both jejunal and ileal sections, showed that the preferential movement for propionic acid was in the jejunum rather than the ileum (55).

For 2 weeks, twelve adult brown leghorn chickens were fed a diet containing approximately 2.5% propionate. Upon examination of the contents of gut sections, it was found that fatty acids were completely removed from the ingesta before it reached Meckel's diverticulum (35).

A study was conducted to determine the nature of the gastric mucosal entry by fatty acid and acetylsalicylic acid in vagally denervated pouches in the oxyntic gland area. Results obtained by 2 to 30 minute periods of irrigation of the denervated pouches with a solution containing 100 millimols of propionic acid revealed that short-chain fatty acids must be in fat soluble form before they

can diffuse through the mucosal barrier, and that once through the barrier, they cause damage to the mucosa (63).

The gastrocnemius muscle of the dog was isolated and transferred to a oxygenated blood circuit for perfusion. Isotopic sodium propionate was injected, and the collection of respiratory CO_2 in sodium hydroxide absorbers was begun. The labeled propionate was injected into the bath at levels of 5, 10, 15, 20, and 25 millimols/1, and the results show that 9 to 13% of the respiratory CO_2 was derived from the carboxyl carbon of propionate in the resting skeletal muscle preparation and only 3-5% in the repetitively contracting gastrocnemius. Evidence was obtained for the conversion of carboxyl-labeled propionate to carboxyl lactate by isolated skeletal muscle but not for its incorporation into muscle glycogen. When acetate and propionate were administered simultaneously to the resting muscle, the rate of conversion of the carboxyl of the propionate to CO_2 was reduced to a much greater extent than that of the carboxyl carbon of acetate (207).

To study the deep depression of feed intake by injected metabolites, goats were fitted with ruminal fistulas and were infused intraruminally for 2 days with 2.5, 5, 10, and 20 millimols/minute of propionic acid. The results showed that feed intake was decreased, but there was no difference between the effect caused by sodium propionate or propionic acid when tested by a paired t-test. Sodium propionate ingestions caused an increase in oral water intake as the rates of sodium injection increased, whereas propionic acid injections caused reduced oral water intake with increasing rate of injections (393).

Holstein and Brown Swiss dairy cows were used to study sodium propionate's effect on milk and fat production, roughage consumption, blood sugar, and blood ketones. The results showed that feeding 1/2 pound of sodium propionate daily to these animals did not affect milk production, hay consumption, blood sugar levels, or weight gains, but there was a slight yet not significant decrease in butter fat. A supplemental study to determine the fatty acid content of rumen fluid was conducted in two fistulated animals fed propionate, and the results showed that there were higher than normal levels of all volatile fatty acids in the rumen for the first 3 hours after feeding. Calculations showed that all of the administered propionate had disappeared in 7 hours (458).

Propionic acid was administered intravenously by constant drip for 8 hours on three consecutive days to study appetite inhibition in dairy cattle by certain intermediate metabolites. Administration of the propionic acid resulted in a significant reduction in intake during 8-hour and 24-hour periods, but there was very little carryover since eating began very soon after cessation of each injection period (72).

When 1.65 millicuries of radio-labeled propionate was infused through a teat cannula into the udder of lactating cows to study the synthesis of milk fat in the bovine mammary gland, the results showed that there was no synthesis of glycerol in the mammary gland caused by the infusion of propionate (215).

Twin Holstein cows and a Guernsey steer were fitted with fistula plugs and used in studies to determine the amounts of short-chain acids formed during rumen fermentation. The concentration of propionic acid in the rumen was determined at various times after feeding either an all-hay ration or one consisting largely of concentrates, and the results revealed that the concentrations of each acid first increased and then decreased after feeding. The data also revealed that the rate of disappearance of propionic acid in high-concentrate rations was 4.6%/hour, and 3.2%/hour in the hay ration (87).

Six female Heidehain pouch dogs with total antrum resections were used to study the stimulation of gastric acid secretion. The results show that propionate administered by venoclysis in a dose of 2.5 to 5.0 μm/kg/minute had a pronounced stimulatory effect on the secretion of gastric acid from the pouches (435).

Rumen abomasal Crocker-Markowitz fistulas, oesophagostomy and venous catherization were performed on 1- to 2-hour-old Clun-Forest sheep to obtain information about factors responsible for initiating and maintaining abomasal secretion. 250 millimols of propionic acid, neutralized to pH 5.5 with sodium hydroxide, were added to rumen contents at hourly intervals; the results show that propionic acid stimulated abomasal secretion (138).

To determine the biological activity of some acids, a cat was injected intravenously with a dose of 1 X 10^{-4} m/kg of sodium propionate. To study the in vivo and in vitro effects, a rat uterus was bathed with a 10^{-6} solution of sodium propionate. The results showed that injected into the cat, sodium propionate caused hypertension and a pressure decrease (about 40 mm of mercury) for 1 minute. It was indicated that sodium propionate had no effect on the profused rat uterus (451).

Merino wethers, 2 to 3 years of age, were infused for 60 minutes through the portal vein with solutions of C_{14}-labeled sodium propionate at the rate of 0.1 microcuries/minute/kg of body weight. Blood samples were drawn from the right jugular vein at termination of the infusion to determine the metabolism of propionic acid. At the end of the infusion, samples of liver and muscle were removed for glycogen assay. The results showed that the specific radioactivity of liver and muscle glycogen was low after the infusion of the labeled propionate. The C_{14}-1-propionate gave rise to labeling mainly in carbon 3 and 4 of glucose and carbon 1 of lactate, while C_{14}-2-propionate labeled mainly carbon 1, 2, 5, and 6 of glucose and carbon 1 and 2 of lactate (8).

Fresian cows fitted with large, permanent rumen fistulas were used in an infusion study of the effect of volatile fatty acid and lactic acid on the secretion of the component fatty acids in milk fat and on blood composition. In the first study, propionic acid

was infused into two separate cows at a rate of 1500 ml/day for one-week periods in order to study the fat content and fatty acid composition of milk. The results showed that during the infusion of propionic acid, palmitic acid was unaltered, but the yield of all other major fatty acids, with the possible exception of lauric acid, was decreased. In the second, third, and fourth series of experiments, propionic acid was infused at a rate of 1600 ml/day for 4 weeks to correlate blood composition as related to the changes observed in milk fat content. The results showed that propionic acid increased the concentration of glucose and decreased that of ketone body. Propionic acid caused only small increases in the volatile fatty acid content of the blood, indicating that the added acids were almost completely metabolized in the rumen epithelium or in the liver (334).

To demonstrate the utilization of propionate for hemesynthesis in human bone marrow cells, normal and megaloblastic bone marrow cells were compared in vitro with respect to the incorporation of C_{14}-labeled propionate. In megaloblastic bone marrow cells obtained from patients with Vitamin B_{12} deficiency, the incorporation of carbon-labeled propionate into heme was markedly reduced. The addition of coenzyme B_{12} specific for a Vitamin B_{12}-deficient state was found to be not directly related to morphologically identified megaloblastic chantes (528).

The absorption of short-chain fatty acids was studied in a patient whose operation had left him with a fistula. The colon was profused with a solution containing acetic, propionic, butyric, isobutyric, valeric, isovaleric and caporic acids in equilmolar amounts. The results show that absorption of short-chain fatty acids from a human's jejunum and colon depends on the chain length; the longer the chain, the faster the acids were absorbed (65).

To study the role of propionic acid as a precursor of methylmalonic acid in normal and Vitamin B_{12}-deficient men, fasting subjects were injected with 10 to 15 microcuries of C_{14}-2-propionate intravenously and/or microcuries of C_{14}-2-methylmalonic acid. In addition, one of the Vitamin B_{12}-deficient patients also received a 5 g oral dose of sodium propionate 30 minutes prior to the i.v. injection of the labeled propionate. The results of the loading study indicated that propionate is a precursor of urinary methylmalonic acid and, therefore, intracellular methylmalonyl CoA in both normal and Vitamin B_{12}-deficient humans. The injected activity found in urinary methylmalonic acid was small, and the turnover studies indicate 20 to 50% is the daily turnover in normal patients and 20% in Vitamin B_{12}-deficient patients. The fate of the remainder is unknown (67).

For 10 days, adult Wistar rats were fed ad libitum a diet composed of 25 g casein, 10 g fat, 51 g dextrose, 3 g salt mixture, 5 g cellulose, 6 g brewers yeast, 0.04 g cod liver oil concentration

and 0.01 g alpha tocopherol. Liver sections were taken to study the pattern alteration of hepatic carbohydrate metabolism by the addition of propionate as a substrate to the tissue bath. The results showed that propionate reduced the $C_{14}O_2$ production to about 53% of that produced in control liver slices, while reducing the incorporation of the C_{14} into lipids to 24% of the control values (95).

Slices of mammary glands and livers of lactating Addis-Sloanaker rats were used to study propionate metabolism using 3, 5, 10, and 50 micromols/incubation vessels of C_{14} propionate. The experiments showed that, in the presence of glucose, C_{14}-1-propionate was extensively incorporated into fatty acids by mammary gland slices. The incorporation of C_{14}-2 and C_{14}-3-propionate into fatty acids was somewhat greater than that of C_{14}-1-propionate. The composition of the fatty acids from all labeled carbons was similar, but those labeled at the -2 and -3 carbon also contained approximately 20% more even carbon acids. The water soluble products formed from propionate C_{14} were determined in mammary glands, and it was found that the major labeled compounds were glutamic acid and glutamine, but when determined in the liver slices, the major products were CO_2 and glucose (169).

The conversion of propionate to liver glycogen in the intact rat was studied using isotopic propionate. Variously labeled isotopic propionate + glucose preparations were fed to fasted rats, and after a suitable period of time, the livers were removed, the glycogen

isolated and hydrolyzed to glucose, and the distribution of isotopes in the glucose determined. The results revealed that the isotopes from the carboxyl group of propionates were found to give detectable labeling only in carbon atoms 3 and 4 of the glucose, while propionate-containing isotopes in the alpha or beta position gave rise to glucose labeled, predominantly and equally, in carbons 1, 2, 5, and 6 (517).

Female goats were employed to determine whether the administration of the individual volatile fatty acids, and combinations thereof, caused changes in the blood glucose and ketone body levels consistent with the generally-accepted mechanism of metabolism. Propionic acid was administered orally by a constant rate pump and passed into the rumen by way of the nostrils. Intravenous administration was made with a constantly-supervised drip device through a plastic tube inserted in the jugular vein. The data obtained from these studies demonstrated that propionic acid counteracts the formation of ketone bodies and causes a marked increase in blood glucose (161).

To study the effects of volatile fatty acids, including propionic acid, on serum insulin concentration, 4 ewes, 2 to 5 years of age, were administered a propionate solution intravenously (neutralized to pH 7 with sodium hydroxide) at a dose of 2.5 millimols/kg. Blood samples were drawn from the jugular vein 30 and 50 minutes before the injection and 0, 5, 10, 20, 30, 40, 60, and 90 minutes after

injection and were analyzed for free fatty acids, glucose, total ketone body, and insulin. The intravenous administration of propionate caused a slight increase in serum insulin, a marked hypoglycemia, and an immediate free fatty acid reduction which lasted for at least 90 minutes after the injection (223).

Presented in the same report was a study of propionate's effect on the changes in pancreatic activity at varying ages. Three sets of twin lambs were administered 2.5 millimols/kg of propionate intravenously either at 12 to 16, 42 to 46, 84 to 88, or 168 to 172 days of age. The administration of propionate produced an elevation in blood glucose and an initially slight hyperglycemia at all ages, but it was most evident in the lambs between 168 and 172 days of age. It was also found that the blood glucose concentrations returned to normal values sooner in the animals that were 12 to 16 days of age (223).

Fasted, adult phlorhizin-treated ewes weighing 130 to 150 pounds were used to study the effects of orally-administered short-chain fatty acids on certain blood and urine constituents. The animals were subjected to a 48-hour fasting period and twice-daily subcutaneous injections of 1g of phlorhizin dissolved in 5 ml of ethylene glycol were given. After this period, fatty acids were administered orally, and blood and urine specimens were collected for a 48-hour period. It was found that when propionic acid was administered in a dose of 1 g-mol/100 pounds of body weight, there was a significant increase

in blood glucose levels at 1, 3, 6, and 24 hours after treatment, with the greatest increase seen at the end of the 3rd hour. The blood ketone level was significantly below the controls, and the urine showed a marked increase in urinary glucose excretion during the 48-hour period after treatment (118).

A chronic surgical preparation permitting local enrichment of the pancreatic arterial blood was used as part of an experiment designed to determine if propionate played an important physiological role in regulating insulin secretion in sheep. When infused at a rate of 0.8 millimols/minute into the jugular vein, propionate produced a rapid and prolonged insulin elevation from 50 to 165 μ/ml. The glucose levels did not change in any of the trials involving propionate (222).

Seven dairy calves, 4 to 7 months of age, were administered 3 ml of P-32-tagged erythrocytes which were injected into the coeliac artery and collected from the gastro-splenic vein. The difference in the amount of fatty acids in blood taken from the jugular vein and that taken from the splenic vein was taken as the measure of absorption. Based on the anlaysis of serial blood samples obtained, it was calculated that 25 g of propionic acid would be absorbed for each pound of dry feed consumed/animal during a 24-hour period (58).

Mixed white-faced lambs, Holstein calves 5 to 90 days old, and mature dogs and horses were used to study the effect of volatile fatty acids on plasma glucose concentrations. The acids, in 2.5

normal solutions neutralized with sodium hydroxide to pH 7.2, were administered intravenously in doses of 2.5 mm/kg of body weight and caused a small plasma glucose increase and a remission of hypoglycemia convulsions in lambs, but did not affect the plasma glucose levels in other species. The small number of animals and the complications involved in administering the compounds made it difficult for the investigators to assess the value of this particular study (275).

Lipid solubility, permeability and hemolytic action of the saturated fatty acids were studied in human and dog red blood cells. The results show that propionic acid was the second most effective of the fatty acids in penetrating the red blood corpuscles, thus producing hemolysis, and that increasing the osmotic concentration of the outside fluid retards hemolysis. Human corpuscles were found to be more resistant to hemolysis by fatty acids than dog corpuscles; the resistance was apparently due to the greater buffer content of washed human corpuscles resulting in the neutralization of a greater amount of acid (32).

In an experiment studying the influence of fatty acid ions on the electro-encephalogram patterns of adult male albino rabbits weighing approximately 2 kg were injected intravenously with a propionate solution at 4 millimols/kg of body weight which immediately induced changes in the EEG patterns from a resting type to one of sleep. This sleep pattern was similar to the EEG pattern observed

when the animals were under deep sodium penta-barbitol anesthesia (372).

The effects of metabolically-important substances on the rabbit auricle were determined by addition of substances to a bath in which the auricles were suspended. Propionates introduced to the bath showed a tendency to induce arrhythmias which were occasionally completely irregular but usually were of the pulus alternas type (amplitude of contractions with each beat regularly alternating between two values). Rapid recovery from these arrhythmias was observed when the propionate was removed from the bath (368).

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